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Synthesis of neosaponins having an α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-D-glucopyranosyl glyco-linkage

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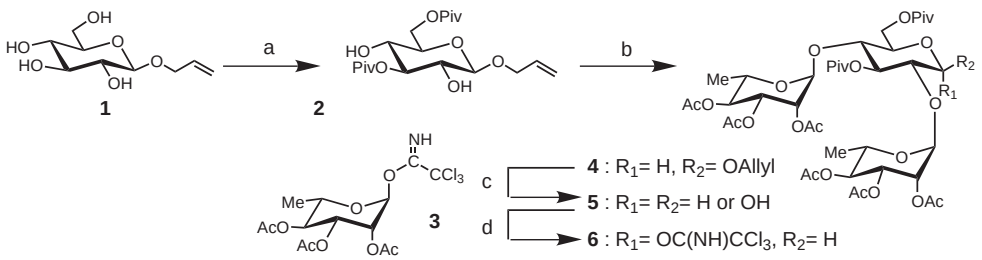
Abstract—To verify the role of the α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl (chacotriosyl) moiety of steroidal glycosides from *Solaum* plants in their antitumor and antivirus activities, chacotriosides of diosgenin, cholesterol, and glycrrhetic acid were synthesized by developing our *trans*-oligoglycosidation. The chacotriosyl trichloroacetimidate was linked to the aglycones to afford neoglycosides as a mixture of α and β anomers, that was easily separated with ODS chromatography.

Recent developments in glycobiology have revealed the important roles of many glycoconjugates in immune responses, viral and bacterial infections, inflammation and many other inter- and intracellular signal transductions. Among the huge number of glycoconjugates, we have been interested in the steroidal glycosides, which are often found as the major components in traditional Chinese medicines, because of their remarkable pharmacological and biological activities.[1] Especially, the steroidal glycosides isolated from *Solanum* plants, e.g. *S. dulcamara*, *S. lyratum*, and *S. nigrum*,[2] exhibited cytotoxicity toward human cancer cells[3] and herpes simplex virus type 1 (HSV-1)[4] in good accordance with the fact that these medicinal plants have been utilized to treat patients suffering from cancer or herpes infection. Our detailed studies on the relationship between the structure and bioactivities of the glycosides from *Solanum* plants showed a potent anti-tumor activity in α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl (chacotriosyl) steroids, while the compounds having other sugar chains were almost inactive.[3] In addition, Kuo reported that the chacotriosyl moiety of solamar-gine (chacotriosyl solasodine), a major steroidal alkaloid from *Solanum* plants, caused triggering cell death by apoptosis.[5] Therefore, we embarked on a synthetic study of several chacotriosyl glycoconjugates to examine their biological activities.



Scheme 1. *Reagents and conditions*: (a) pivaloyl chloride, pyridine, 0°C for 3 h, 68%; (b) **3**, MS4A, CH_2Cl_2, N_2, −78°C, 1 h, 89%; (c) $Pd[P(Ph)_3]_4$, AcOH, 80°C, 71%; (d) CCl_3CN, DBU, CH_2Cl_2, 0°C, 2 h, 75%.

Keywords: neosaponin; glycoconjugate; *trans*-oligoglycosidation; *Solanum* plant; chacotriose.

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We have reported the *trans*-oligoglycosylation of bioactive glycolinkages from natural glycosides to various aglycones.[6] This method is advantageous in the case where the oligoglycosyl moiety transferred is naturally abundant and/or easily prepared on a large scale. Applying this method to the synthesis of a series of neo-chacotriosides (one of neosaponins), we prepared allyl chacotrioside as the original glycoside and transformed to chacotriosyl trichloroacetimidate as the glycosyl donor in the first step. In the next step, the trichloroacetimidate is activated with a Lewis acid to form the glycosyl linkage with several aglycones. In order to investigate the bioactivity of the synthesized neosaponins in comparison with the library of steroidal glycosides isolated from *Solanum* plants in our laboratory, we chose diosgenin, cholesterol, and glycyrrhetinic acid as the aglycone moieties.

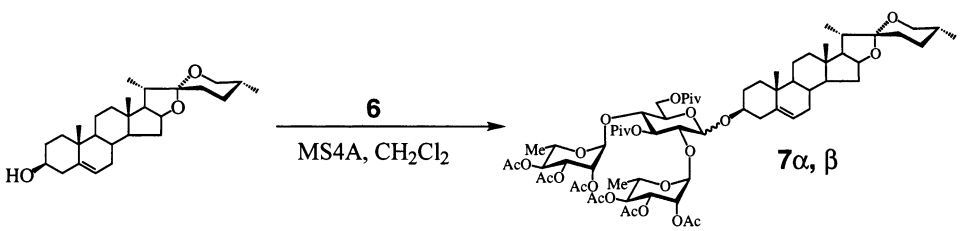
Allyl β-D-glucopyranoside **1**[7] was selectively protected with pivaloyl chloride in pyridine at 0°C to give an allyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside **2**[8] (Scheme 1). The 2 and 4 positions of the glucose donor **2** were glycosylated with 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl trichloroacetimidete **3**[9a] using boron trifluoride etherate ($BF_3{\cdot}Et_2O$)[9] as the promoter to give fully protected chacotrioside **4**. The trisaccharide **5** was converted to an α-trichloroacetimidate **6**[10] by the deallylation with $Pd[P(Ph)_3]_4$.[11]

The trisaccharide donor **6** was transferred to diosgenin by Schmidt's 'inverse procedure',[12] in which the hydroxyl group of the acceptor was activated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) due to the steric hindrance of 3-OH in steroids and triterpenoids, and the fully protected glycoside **7** was obtained as a mixture of α and β anomers (1:0.24, α-anomer; δ 4.93, d, $J=3.7$ Hz, β-anomer; δ 4.47, d, $J=7.3$ Hz) in 59% yield (Table 1, entry 1).

However, stereoselectivity of the reaction was not satisfactory to afford the β anomer, so that several conditions were examined (Table 1, entries 2–8). In the beginning, the reaction temperature and Lewis acid were, respectively changed to room temperature (entry 2) and to $BF_3{\cdot}Et_2O$ (entry 5); however, the ratio of the β anomer was not increased. This result suggested that the glycosylation reaction was controlled thermodynamically via an Sn1-type reaction; therefore, the glycosylation was attempted under a 'normal procedure', i.e. adding the Lewis acid to a solution of diosgenin and **6** in CH_2Cl_2 (entries 3, 4, 6–8). A catalytic amount of TMSOTf was used in entries 3 and 4. Entry 4 reacted at 22°C showed an increase of both β selectivity and the chemical yield, while entry 3 was not changed selectivity as well as in the 'inverse procedure'. When an excess amount of $BF_3{\cdot}Et_2O$ was employed (entries 6 and 7), the ratio of β anomer was the most increased in all the cases. On the other hand, when a catalytic amount of $BF_3{\cdot}Et_2O$ was employed (entry 8), the yield of glycosylation was best, but the β selectivity was similar to that in entry 4. These results suggested that relatively high reaction temperature is effective to give the high β selectivity and the chemical yield (entries 3, 4, 6, 7). Probably, kinetically controlled glycosylation was slightly increased owing to the higher reaction temperature. Furthermore, the excess amounts of $BF_3{\cdot}Et_2O$ under the 'normal procedure' increased β stereo selectivity. The glycosyl donor **6** might be converted to a highly reactive carbenium-ion intermediate, and then the excess amounts of $BF_3{\cdot}Et_2O$ could be coordinated at the thermodynamically stable α face of the intermediate, and S_N2 reaction took place to increase β selectivity.

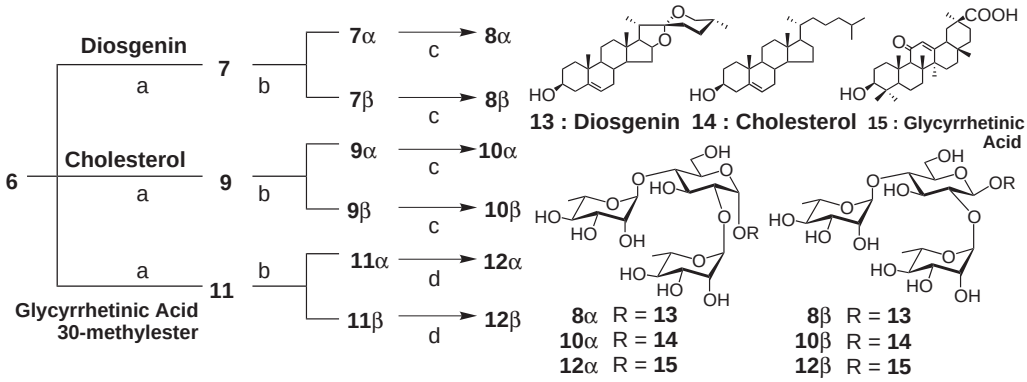
Therefore, we chose the conditions shown in entry 7 to synthesize other neosaponins. Cholesterol and glycyrrhetinic acid methyl ester were glycosylated with **6** in

Table 1. Results of glycosylation of **6** and diosgenin



Entries		Lewis acid (equiv.)	Temp (°C)	[α:β]	Yield (%)
1	I.P.[a]	TMSOTf (3.2)	0	1:0.24	58.3
2	I.P.[a]	TMSOTf (2.2)	−78→r.t.	1:0.28	63.4
3	N.P.[b]	TMSOTf (0.01)	−78→r.t.	1:0.26	68.4
4	N.P.[b]	TMSOTf (0.01)	22	1:0.35	72.1
5	I.P.[a]	$BF_3{\cdot}Et_2O$ (12)	0	1:0.25	53.5
6	N.P.[b]	$BF_3{\cdot}Et_2O$ (12)	0	1:0.43	60.2
7	N.P.[b]	$BF_3{\cdot}Et_2O$ (11)	20	1:0.44	64.3
8	N.P.[b]	$BF_3{\cdot}Et_2O$ (0.05)	20	1:0.36	81.5

[a] I.P. = inverse procedure.
[b] N.P. = normal procedure.



Scheme 2. *Reagents and conditions*: (a) $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt; (b) ODS (90% MeOH); (c) 3% KOH/MeOH, reflux, 92–97%; (d) 3% LiOH/MeOH, reflux, 80–85%.

the same manner described above (entry 7) to afford an α and β anomer mixture of chacotriosyl cholesterol **9** (55.8%, α:β = 1:0.73) and chacotriosyl glycyrrehetic acid **11** (52.6%, α:β = 1:0.56), respectively. The anomeric mixtures of α- and β-chacotriosides were separated by octadodecyl silica gel (ODS) column chromatography using 90% MeOH to give α and β chacotriosides (**7**α, **7**β, **9**α, **9**β, **11**α, and **11**β), respectively. Each glycoside separated by ODS was deprotected in the usual manner to give **8**α, **8**β, **10**α, **10**β, **12**α, and **12**β[13] (Scheme 2).

The cytotoxicity of the obtained neosaponins (**8**α, **8**β, **10**α, **10**β, **12**α, and **12**β) was tested with HCT116 and PC-12 cell lines. Compound **8**β showed cytotoxicity {IC_{50}: 2.66 μg/mL (HCT116); 1.99 μg/mL (PC-12)}, while the other neosaponins showed no cytotoxicity (>5 μg/mL).

In conclusion, facile preparation of an α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-D-glucopyranosyl (chacotriosyl) trichloroacetimidate (**6**) has been achieved (from the allyl glucoside to **6** by 4 steps in 32% overall yield) in spite of the fact that the α glycosides were more predominant than the β glycosides in the oligoglycosylation. Since only the **8**β showed cytotoxicity, both the β stereochemistry of chacotrioside and the steroidal aglycone moiety are crucial for the cytotoxicity. To investigate futher structure-activity relationship, synthesis of more β-chacortiosyl derivatives and bioassays are now in progress.

Acknowledgements

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References

1. *Studies in Plant Science*, 6; Yang, C.-R.; Tanaka, O., Eds.; Advances in plant glycosides, chemistry and biology. Elsevier: Amsterdam, 1999.
2. (a) *Encyclopedia of Contemporary Chinese Medical Plants*; Chi, S. Ed. (translation editor, Sugi, M.); Kogyo Chosakai: Tokyo, 1980; pp. 79–87; (b) *Chinese Drug Dictionary*; Koso New Medical College, Ed., Shanghai Science and Technology Publishing Co.: Shanghai, 1978; Vol. 1, pp. 630–631.
3. Nakamura, T.; Komori, C.; Lee, Y.-Y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564–566.
4. Ikeda, T.; Ando, J.; Miyazono, A.; Zhu, X.-H.; Tsumagari, H.; Nohara, T.; Yokomizo, K.; Uyeda, M. *Biol. Pharm. Bull.* **2000**, *23*, 364–365.
5. (a) Hsu, S.-H.; Tsai, T.-R.; Lin, C.-N.; Yen, M.-H.; Kuo, K.-W. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 1–5; (b) Chang, L.-C.; Tsai, T.-R.; Wang, J.-J.; Lin, C.-N.; Kuo, K.-W. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 21–25.
6. (a) Ikeda, T.; Kajimoto, T.; Kinjo, J.; Nakayama, K.; Nohara, T. *Tetrahedron Lett.* **1998**, *39*, 3513–3516; (b) Ikeda, T.; Kinjo, J.; Kajimoto, T.; Nohara, T. *Heterocycles* **2000**, *52*, 775–798.
7. Compound **1** was readily prepared from 1,2,3,4,6-penta-*O*-acetyl-D-glucose and allylalchohol in the presence of $BF_3{\cdot}Et_2O$. (a) Ogawa, T.; Beppu, K.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, C6; (b) Ferrier, R. J.; Furneaux, R. H. *Methods Carbohydr. Chem.* **1980**, *8*, 251.
8. (a) Jiang, L.; Chan, T.-H. *J. Org. Chem.* **1998**, *63*, 6035–6038; (b) Kurahashi, T.; Mizutani, T.; Yoshida, J. *J. Chem., Soc. Perkin Trans. 1* **1999**, 465–473.
9. (a) Schmidt, R. R. *Advan. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123; (b) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *39*, 731–732.
10. Compound **6**: $[\alpha]_D^{25}$ +16.0° (*c* 0.43, $CHCl_3$); δ_H ($CDCl_3$, 500 MHz): 1.15 (3H, d, *J* = 6.7 Hz, rha-6), 1.17 (3H, d, *J* = 6.1 Hz, rha-6), 1.21, 1.23 (18H, s, Piv), 1.97, 1.98, 2.04×2, 2.12×2 (18H, s, acetyl), 4.81 (1H, br s, rha-1), 4.91 (1H, d, *J* = 1.8 Hz, rha-1), 5.65 (1H, dd, *J* = 9.2, 9.8 Hz, glc-3), 6.42 (1H, d, *J* = 3.7 Hz, glc-1α), 8.77 (1H, s, -NH); FABMS (positive) *m*/*z* 1058 $(M+Na)^+$.
11. Nakayama, K.; Uoto, K.; Higashi, K.; Soga, T.; Kusama, T. *Chem. Pharm. Bull.* **1992**, *40*, 1718–1720.
12. Schmidt, R. R.; Toepfer, A. *Tetrahedron. Lett.* **1991**, *32*, 3353–3356.
13. Selected spectroscopic data: **8**α: $[\alpha]_D^{30}$ −26.1° (*c* 0.11, MeOH); δ_H (C_5D_5N): 0.70 (3H, d, *J* = 5.5 Hz, H-27),

0.84, 0.88 (each 3H, s, H-18, 19), 1.15 (3H, d, $J=6.7$ Hz, H-21), 1.68, 1.70 (each 3H, d, $J=6.1$ Hz, rha-6×2), 5.33 (1H, br s, H-6), 5.52 (1H, d, $J=3.7$ Hz, glc-1), 5.86 (1H, s, rha-1), 5.95 (1H, s, rha-1); FABMS (positive) m/z 891 $(M+Na)^+$. **8**β: $[\alpha]_D^{30}$ −102.0° (*c* 0.43, MeOH); δ_H (C_5D_5N): 0.71 (3H, d, $J=5.5$ Hz, H-27), 0.84, 1.06 (each 3H, s, H-18, 19), 1.14 (3H, d, $J=7.3$ Hz, H-21), 1.63, 1.77 (each 3H, d, $J=6.1$ Hz, rha-6×2), 4.95 (1H, d, $J=7.9$ Hz, glc-1), 5.33 (1H, br s, H-6), 5.85 (1H, s, rha-1), 6.40 (1H, s, rha-1); FABMS (positive) m/z 891 $(M+Na)^+$. **10**α: $[\alpha]_D^{30}$ +5.5° (*c* 0.11, MeOH); δ_H (C_5D_5N): 0.66 (3H, s, H-18), 0.91 (6H, d, $J=6.1$ Hz, H-26, 27), 0.91 (3H, s, H-19), 0.98 (3H, d, $J=6.1$ Hz, H-21), 1.69, 1.71 (each 3H, d, $J=6.1$ Hz, rha-6×2), 5.36 (1H, br s, H-6), 5.53 (1H, d, $J=3.7$ Hz, glc-1), 5.87 (1H, s, rha-1), 5.97 (1H, s, rha-1); FABMS (positive) m/z 863 $(M+Na)^+$. **10**β: $[\alpha]_D^{30}$ −42.2° (*c* 0.10, MeOH); δ_H (C_5D_5N) 0.66 (3H, s, H-18), 0.90, 0.91 (each 3H, d, $J=6.7$ Hz, H-26, 27), 0.97 (3H, d, $J=6.7$ Hz, H-21), 1.08 (3H, s, H-19), 1.64, 1.78 (each 3H, d, $J=6.1$ Hz, rha-6×2), 4.96 (1H, d, $J=7.9$ Hz, glc-1), 5.37 (1H, br s, H-6), 5.86 (1H, s, rha-1), 6.40 (1H, s, rha-1); FABMS (positive) m/z 863 $(M+Na)^+$. **12**α: $[\alpha]_D^{30}$ +56.5° (*c* 0.13, MeOH); δ_H (C_5D_5N): 0.98, 1.08, 1.13, 1.24, 1.30, 1.33, 1.38 (each 3H, s, H-23–H-29), 1.63 (6H, d, $J=6.1$ Hz, rha-6×2), 5.49 (1H, br s, glc-1), 5.85 (1H, s, rha-1), 6.01 (1H, s, rha-1), 6.44 (1H, br s, H-12); FABMS (positive) m/z 947 $(M+Na)^+$. **12**β: $[\alpha]_D^{30}$ +27.8 ° (*c* 0.10, MeOH); δ_H (C_5D_5N): 1.09, 1.23, 1.25, 1.28, 1.30, 1.34, 1.43 (each 3H, s, H-23∼29), 1.59, 1.70 (each 3H, d, $J=6.1$ Hz, rha-6×2), 4.91 (1H, d, $J=7.9$ Hz, glc-1), 5.86 (1H, s, rha-1), 6.00 (1H, s, rha-1), 6.61 (1H, br s, H-12); FABMS (positive) m/z 1058 $(M+Na)^+$.